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Integrative systematics of a new species of the gall wasp genus *Belonocnema* Mayr (Cynipidae, Cynipini) on the Mesoamerican live oak *Quercus oleoides* (Fagaceae) and its associated hymenopteran community

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Abstract

Belonocnema Mayr (Cynipidae, Cynipini) is an oak gall wasp genus currently known by three described species distributed in the southern and southeastern U.S. and northern Mexico, all associated with live oaks of the *Quercus* section Virentes. Here, we conducted an integrative systematic study of a new species of *Belonocnema*, *B. mesoamericana* Zhang, Zaldívar-Riverón & Fernández-Flores, **sp. nov.**, reared from galls of the live oak *Quercus oleoides* Schltdl. in the state of Veracruz, Mexico. We morphologically described the sexual and asexual generations of the new species and their galls, molecularly characterized it based on the DNA barcoding locus, reconstructed its phylogenetic affinities using multilocus data from Ultraconserved Elements, and identified its associated hymenopteran community. The new species is morphologically similar to its sister species, *B. kinseyi* Weld, though it can be distinguished by color and sculpture features, as well as by the sexual generation forming velvety rootlet galls. Since *Q. oleoides* is widely distributed across Mesoamerica, we expect the new species to have a broader distribution. A con-

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siderably high species richness and phylogenetic diversity of the hymenopteran community associated with the galls were recovered, with 43 morphospecies belonging to 15 genera and 13 families, of which 41 were found in the asexual generation and only three in the sexual generation. This study increases the systematic knowledge of a poorly known Cynipini genus and highlights the extraordinary, yet neglected, insect diversity associated with galls, especially in the Neotropics.

Keywords
DNA barcoding, Hymenoptera, Mexico, Neotropics, Ultraconserved Elements

Introduction

With over 1000 described species, the tribe Cynipini represents one of the largest phytophagous lineages both within the family and the entire order Hymenoptera, being composed exclusively of gall-forming species (Ronquist et al. 2015). Most members of Cynipini induce galls on oaks of the genus *Quercus* L.; however, some species induce galls on other Fagaceae genera such as *Castanea* Mill., *Castanopsis* (D. Don) Spach, *Chrysolepis* Hjelmq., and *Lithocarpus* Blume (Stone et al. 2002). Members of this genus are distributed in the Americas and Europe, mainly in temperate and tropical forests in the northern hemisphere, with around 560–580 valid species worldwide (PlantaeDB 2026). Currently, *Quercus* is divided in two subgenera, *Quercus* and *Cerris*, which are further subdivided in eight formal sections (Denk et al. 2017).

Live oaks (*Quercus* section Virentes) are a group of seven species that occur in both temperate and coastal dry regions. Four species are distributed in the southern and southeastern U.S.: *Q. fusiformis* Small, which also extends into northern Mexico; *Q. geminata* Small; *Q. minima* (Sarg.) Small; and *Q. virginiana* Mill. Moreover, one species occurs in Cuba, *Q. sagraeana* Nutt.; one in Baja California Sur, Mexico, *Q. brandegeei* Goldman; and the last one, *Q. oleoides* Schltdl., has a wide distribution from northern Mexico to Costa Rica in Central America (Valencia 2004; Pennington and Sarukhán 2005; Rzedowski 2006; Cavender-Bares et al. 2015). Current knowledge of Cynipini taxa associated with live oaks is mainly available for the U.S. species, with 24 species that have been described in seven recorded genera (Table 1) (Weld 1959; Melika et al. 2021; Zhang et al. 2021; Brandão-Dias et al. 2022; Cuesta-Porta et al. 2022, 2024). In contrast, for the remaining three *Quercus* species in the section Virentes, only four Cynipini species belonging to two genera have been described to date for *Q. oleoides* – *Disholcaspis cinerosa* (Bassett), *Druon hansoni* Cuesta-Porta, Melika & Pujade-Villar, *Dr. linaria* Kinsey, and *Dr. quercuslanigerum* (Ashmead) (Cuesta-Porta et al. 2022, 2024).

Belonocnema Mayr is a small Cynipini wasp genus with three valid species that are restricted to the oak section Virentes (Weld 1921; Zhang et al. 2021) (Fig. 1A–C): *B. fossoria* Weld, from *Q. geminata*; *B. treatae* Mayr, from eastern populations of *Q. virginiana*, and *B. kinseyi* Weld, from western populations of *Q. virginiana* and *Q. fusiformis*. The galls of the asexual generation of these species are detachable, pea-shaped, unilocular, and are formed on the adaxial surface of the leaves, whereas those from the sexual generation are multilocular, irregularly shaped and located in rootlets just below

Table 1. List of Cynipini species that are known to induce galls on *Quercus* species of the section Virentes. Known generation: S = sexual; A = asexual (source: https://www.gallformers.org).

Cynipini species	Oak species	Known generation
Andricus highlandensis Melika, Nicholls & Stone	*Q. fusiformis*, *Q. geminata*, *Q. virginiana*	S
Andricus quercusfoliatus Ashmead	*Q. fusiformis*, *Q. geminata*, *Q. minima*, *Q. virginiana*	A
Bassettia pallida Ashmead	*Q. geminata*, *Q. minima*, *Q. virginiana*	A
Belonocnema fossoria Weld	*Q. geminata*, *Q. minima*	S, A
Belonocnema kinseyi Weld	*Q. fusiformis*, *Q. virginiana*	S, A
Belonocnema treatae (Mayr)	*Q. virginiana*	S, A
Belonocnema mesoamericana sp. nov.	*Q. oleoides*	S, A
Callirhytis quercusbatatoides (Ashmead)	*Q. fusiformis*, *Q. geminata*, *Q. minima*, *Q. virginiana*	S, A
Disholcaspis cinerosa (= *pallens*) (Basset)	*Q. fusiformis*, *Q. virginiana*	S, A
Disholcaspis fungiformis Kinsey	*Q. fusiformis*	A
Disholcaspis quercusvirens (Ashmead)	*Q. geminata*, *Q. minima*, *Q. virginiana*	S, A
Druon hansoni Pujade-Villar	*Q. oleoides*	A
Druon linaria Kinsey	*Q. oleoides*	A
Druon quercuslanigerum (Ashmead)	*Q. fusiformis*, *Q. geminata*, *Q. minima*, *Q. virginiana*, *Q. oleoides*	S, A
Loxaulus maculipennis (Ashmead)	*Q. virginiana*	A
Loxaulus pattersoni Kinsey	*Q. geminata*, *Q. virginiana*	A
Loxaulus virginianae Melika & Buss	*Q. fusiformis*, *Q. geminata*, *Q. virginiana*	A
Neuroterus bussae Melika & Nicholls	*Q. fusiformis*, *Q. virginiana*	S
Neuroterus christi Melika & Abrahamson	*Q. geminata*, *Q. virginiana*	S
Neuroterus howertoni Kinsey	*Q. fusiformis*, *Q. virginiana*, other non-Virentes oak species	A
Neuroterus niger var. *alimas* Kinsey	*Q. virginiana*	S
Neuroterus quercusminutissimus Kinsey	*Q. geminata*, *Q. minima*, *Q. virginiana*	A
Neuroterus saltatorius texanus Kinsey	*Q. fusiformis*, *geminata*, *Q. virginiana*	A
Neuroterus valhalla Brandao-Dias et al.	*Q. fusiformis*, *Q. virginiana*	S, A
Odontocynips nebulosa Kieffer	*Q. fusiformis*, *geminata*, *Q. minima*, *Q. virginiana*	S, A

the soil surface (Weld 1921; Zhang et al. 2021). A detailed population genomic analysis within the genus *Belonocnema* in the U.S. found strong differentiation associated with host plant use in the eastern part of their range on *Q. geminata* and *Q. virginiana*, and a more complicated network of relationships in the western part of their range on *Q. virginiana* and *Q. fusiformis* (Driscoe et al. 2019). Building from that work, a more recent integrative taxonomic study of *Belonocnema* based on morphological evidence as well as mitochondrial (COI) and nuclear RADseq-derived SNP data, Zhang et al. (2021) resolved the taxonomic status of the above three species, morphologically differentiating their sexual and asexual generations, disentangling their natural history information, and confirming the presence of mito-nuclear discordance between *B. fossoria* and *B. treatae*. However, although this update helped improve the systematics of *Belonocnema*, this effort was restricted to the species that occur in the live oaks distributed in the U.S., leaving the status of the potential species that occur in the remaining live oak species unclear.

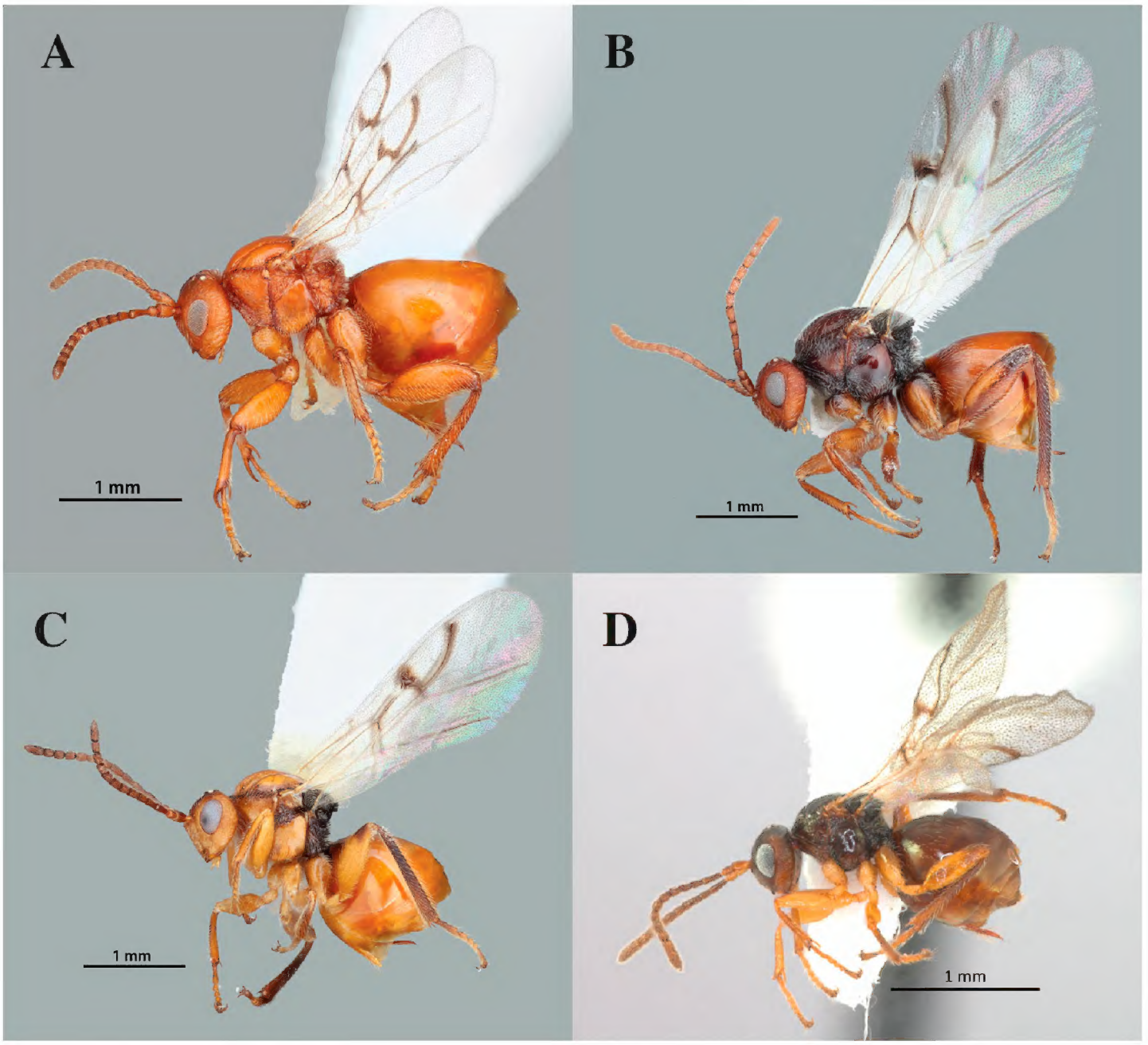

Figure 1. Digital images of female specimens of the asexual generation belonging to the four known *Belonocnema* species (lateral view). **A.** *B. fossoria*; **B.** B. *kinseyi*; **C.** *B. treatae*; **D.** *B. mesoamericana* sp. nov., specimen with darker color.

In recent years, we have reared specimens of *Belonocnema* from pea-shaped leaf galls present on *Q. oleoides* in various localities in the state of Veracruz, Mexico, and their examination revealed that they belong to an undescribed species. Here, we conducted a comprehensive integrative systematic study of this new species, providing a morphological description of its agamic and sexual generations and their galls, molecularly characterizing it based on the DNA barcoding locus (Hebert et al. 2003), and generating multilocus nuclear data using Ultraconserved Elements (UCE, Faircloth et al. 2012) for the four recognized species of *Belonocnema* to reconstruct their phylogenetic relationships. We also characterized the hymenopteran community associated with the galls formed by the two generations of the new species. We found a considerably high hymenopteran species richness and phylogenetic diversity, which is mainly concentrated in galls of the asexual generation.

Materials and methods

Examined material

We carried out a total of eight field trips to a locality near the town of Los Otates, in the municipality of Actopan, state of Veracruz, Mexico, where we found a relatively large population of *Q. oleoides* trees. During the last field trip, we also visited several localities in the vicinity of Xalapa, Veracruz, where we found small populations of this oak species. Field trips were conducted during different seasons between 2018 and 2025, with an emphasis on October–November and March–April. During these trips, we searched for galls on different organs of the oaks, including roots, stems, leaves, catkins, acorns and buds. We collected 11 different gall types, including pea-shaped leaf galls during October and November, and root galls in April. Galls were placed in separate plastic bags according to their type to prevent cross-contamination among insects emerging from each sample. All emerging insects were reared in the laboratory at the Colección Nacional de Insectos (CNIN), Instituto de Biología, UNAM (IBUNAM), and placed in 95% ethanol at -20 °C.

For the description of the new species of *Belonocnema*, we followed Melika (2006) and Buffington et al. (2020) for terminology on Cynipidae morphological structures and abbreviations for fore wing venation, and Harris (1979) for patterns of cuticular sculpture. Digital images of the new species were taken at the Laboratorio Nacional de Biodiversidad (LANABIO) at IBUNAM using a ZEISS® AXIO ZoomV16 stereoscopic microscope, an AxioCam MRc5 (5 megapixels) camera, and the ZEN 2012 (Blue Edition) software. Digital images of the previously described species were taken at the Smithsonian National Museum of Natural History using the Macropod System. The remaining reared hymenopterans were counted, sorted to morphospecies and identified using the relevant literature. All galls and their reared insects, including the type specimens of the new *Belonocnema* species, were deposited in the CNIN-IBUNAM.

Laboratory procedure

Genomic DNA was extracted from four ethanol-preserved specimens of the new species, two from the sexual and two for the asexual generations (DNA voucher numbers CNIN5137–38, 5391–92, respectively) using a non-destructive technique (Ceccarelli et al. 2012) with the EZ-10 Spin Column Genomic DNA minipreps Kit (BioBasic®, Toronto, Ontario, Canada) following the manufacturer's protocol. Digestion was carried out overnight, specimens were then removed, washed in distilled water to eliminate residual proteinase K, and remounted. DNA quantification was performed using a Qubit 4.0 fluorometer and the High Sensitivity kit (Invitrogen, Life Technologies, Carlsbad, California, USA).

Genomic shotgun libraries were prepared using the Illumina DNA Prep Kit (Illumina, San Diego, California, USA) according to the manufacturer's protocol, but

reducing reaction volumes to one-quarter, and indexed using IDT for Illumina UD adapters for *in silico* demultiplexing. Library preparation was performed using up to 100 ng of extracted DNA resuspended in 7,5 µL of ultrapure water. Input DNA was tagmented at 55 °C for 15 minutes to generate fragments with a mean size of 200–500 bp. Generated libraries were quantified with a Qubit 4.0 fluorometer and the High Sensitivity kit (Invitrogen, Life Technologies, Carlsbad, California, USA) and pooled at equimolar concentrations for sequencing.

Three of the above samples (CNIN5137–38, 5392) were processed for target enrichment of UCEs. Genomic libraries for these samples were pooled at equimolar concentrations in a group of 10 libraries, with ~100 ng of DNA each for enrichment reaction. UCE enrichment was performed using the RNA probe set Hym v.2 designed for Hymenoptera (Branstetter et al. 2017), which includes 31,829 baits targeting 2,590 UCE loci, following a combination of the MYbaits protocol ver. 4.01 for enrichment day one, and the original, less stringent UCE protocol for day two (www.ultraconserved.org). Post-enrichment pools were quantified using the Qubit 4.0 fluorometer with the Broad Range Kit (Invitrogen®, Life Technologies, Carlsbad, California, USA). Sequencing for both enriched and unenriched libraries was conducted on an Illumina NovaSeq X Plus instrument (PE150, v4 chemistry) at the Admera Health BioPharma Services (South Plainfield, New Jersey, USA). Raw sequence data are available in the NCBI Sequence Read Archive (NCBI-SRA) under BioProject accession number PRJNA1427309.

Bioinformatic processing

Raw reads for the enriched and unenriched samples were filtered and trimmed using the Trimmomatic tool (ver. 0.39; Bolger et al. 2014). Clean reads were subsequently assembled using the SPAdes tool (ver. 3.15.0; Bankevich et al. 2012), both implemented on the Galaxy open-source web-server platform (see https://usegalaxy.org).

COI Barcode sequences (658 bp) were recovered for three specimens (CNIN5138, 5391–92; GenBank accession nos PZ044865–67) of the new species by mapping the assembled contigs of the unenriched samples to a *B. treatae* barcode sequence (GenBank accession no MG252453; Schuler et al. 2018) that was used as reference in Geneious ver. 10.0.7 (https://www.geneious.com; Kearse et al. 2012). Uncorrected pairwise genetic distances between 24 published sequences of the three previously described species of *Belonocnema* from Schuler et al. (2018; GenBank accession nos MG252389–92, 94–96, 403, 13, 17, 18, 20, 30, 51, 52, 56, 58, 60–62, 65, 67, 69) and the three sequences of the new species were obtained using the program MEGA11 (Tamura et al. 2021).

We used the Phyluce v1.7.4 pipeline (Faircloth 2015) to process the contigs obtained for the three enriched samples of the new species along with four published UCE assemblies from Ward et al. (2022a), which include *B. fossoria*, *B. treatae*, and *Xanthoteras eburneum* (Bassett), using the latter as outgroup to root the tree (Ward et al. 2022a). Additionally, we harvested the *B. kinseyi* genome (GCF_010883055.1) following the Phyluce Tutorial III for UCE loci, with a relaxed setting of—coverage 80

and—identity 80 for phyluce_probe_run_multiple_lastzs_sqlite in order to increase the number of loci recovered. Contigs were assembled using SPAdes ver. 3.14.0 (Prjibelski et al. 2020) and matched against the UCE Hymenoptera 2.5Kv2P (Branstetter et al. 2017) probe set. The assemblies were aligned using MAFFT v 7.490 (Katoh et al. 2002; Katoh and Toh 2008) and trimmed using GBlocks (Castresana 2000) with the following settings: b1 = 0.5, b2 = 0.5, b3 = 12, b4 = 7.

UCE-based phylogenetic analyses

We selected the 70% complete matrix, which represent loci that are present in ≥ 70% of the taxa. We conducted a phylogenetic analysis under the maximum-likelihood criterion with IQ-TREE ver. 2.3.1 (Minh et al. 2020). To minimize the effects of phylogenetic heterogeneity, we filtered the matrices with the symmetry test of stationarity, reversibility and homogeneity (SRH) for sequence alignments (Naser-Khdouret al. 2019). The best model for each locus was selected using ModelFinder v2.0 (Kalyaanamoorthy et al. 2017) with the IQ-TREE command '-MFP + MERGE'. To assess support, we performed 1000 Ultrafast Bootstrap (UFBoot; Hoang et al. 2018), along with '-bnni' to reduce the risk of overestimation, and a Shimodaira–Hasegawa approximate likelihood-rate test (SHaLRT, Guindon et al. 2010) with 1000 replicates. Only nodes with support values of UFB ≥ 95 and SH-aLRT ≥ 80 were considered robust.

Results

DNA barcoding characterization and UCE-based phylogenetic relationships

The uncorrected pairwise distances for the barcoding locus among the three *Belonocnema* specimens of both generations collected in Veracruz, Mexico, ranged from 0.3 to 0.5%. In contrast, pairwise distances between these specimens and *B. kinseyi* ranged from 1.4 to 2.1%, whereas distances to the remaining two species, *B. fossoria* and *B. treatae*, were considerably higher, ranging from 4.7 to 6.3%.

The three newly sequenced samples of the new species of *Belonocnema* (DNA voucher nos CNIN5137, 5138, and 5391) recovered 342, 350, and 298 loci, respectively. The resulting UCE 70% matrix with seven terminal taxa, including the outgroup, consisted of 979 loci. The SRH test removed 87 loci, resulting in a total of 892 loci. The reconstructed phylogram (Fig. 2) recovered the new species of *Belonocnema* as sister to *B. kinseyi* with strong support (100/100 UFBoot/SH-aLRT), with these two species being sister to *B. treatae* + *B. fossoria*.

Based on the external morphological differences found for the examined specimens, together with their genetic divergence at the barcoding locus and its 'exclusivity' (*sensu* de Queiroz, 2007) recovered in the phylogenetic analysis of genomic-scale nuclear data, we describe below the populations of *Belonocnema* from Veracruz, Mexico, as a new species.

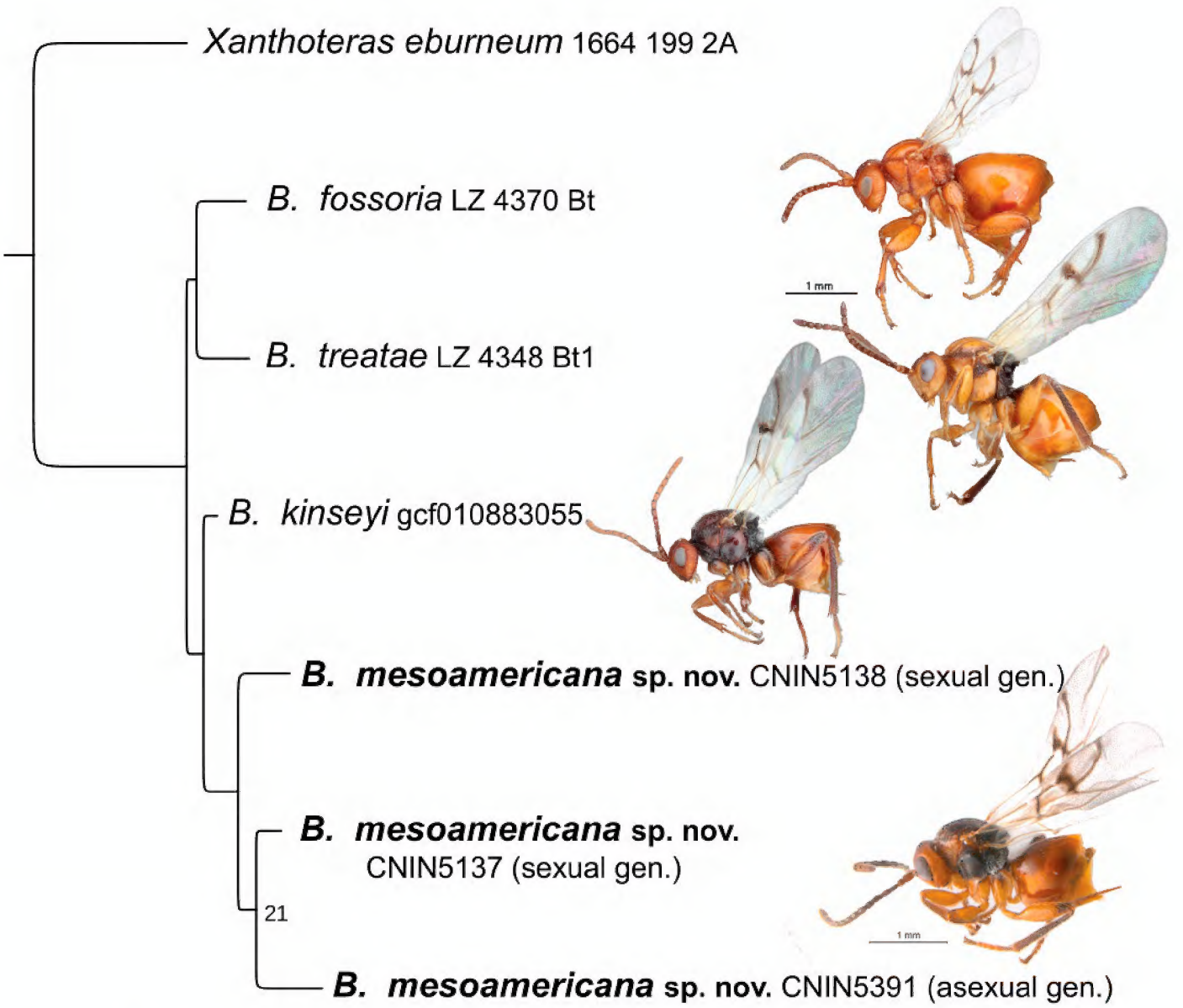
Xanthoteras eburneum 1664 199 2A
B. fossoria LZ 4370 Bt
B. treatae LZ 4348 Bt1
B. kinseyi gcf010883055
B. mesoamericana sp. nov. CNIN5138 (sexual gen.)
B. mesoamericana sp. nov.
CNIN5137 (sexual gen.)
21
B. mesoamericana sp. nov. CNIN5391 (asexual gen.)
1 mm
1 mm

Figure 2. ML phylogram with members of the four species of *Belonocnema*. The number above a node indicates its bootstrap support value; all remaining nodes have a bootstrap support value of 100. Females of the sexual generation belonging to the four species are shown on the right.

Taxonomic part

***Belonocnema mesoamericana* Zhang, Zaldívar-Riverón & Fernández-Flores, sp. nov.**
https://zoobank.org/FBF6D764-B128-4CFB-8908-FD9D7069D74D
Figs 1D, 3A–F, 4A–F

Type material. ***Holotype.*** Asexual generation. Mexico • Veracruz, Municipio Actopan, Otates; 19°30'42.44"N, 96°42'59.98"W; 12/IV/2024; A. Zaldívar, P. Goncalves, cols.; ex. root galls Q. *oleoides*.

Paratypes. Asexual generation: 27 specimens • Twelve females; México, Veracruz, Xalapa, Chavarrillo; 17/X/2025; 19°25'12.12"N, 96°46'48"W; manual; 900 m; A. Zaldívar, L. Rios, A. Rangel, D. Alemeda, J. Solis, cols.; *Quercus oleoides*, agalla IV, *Belonocnema* sp., haz de hoja, múltiples semillas, rojizas • Six females; México, Veracruz, Actopan, "Los Otates"; 22/XI/2019; 19°30'18.7"N, 96°43'16.7"W; manual; 840 m; A. Zaldívar, J. Solis, S. Fernández, cols.; *Quercus oleoides*, agalla IV, *Belonocnema* sp., haz de hoja, múltiples semillas, rojizas • Seven females; Los Otates, sitio #1; 22/XI/2019; 19°30'48.2"N,

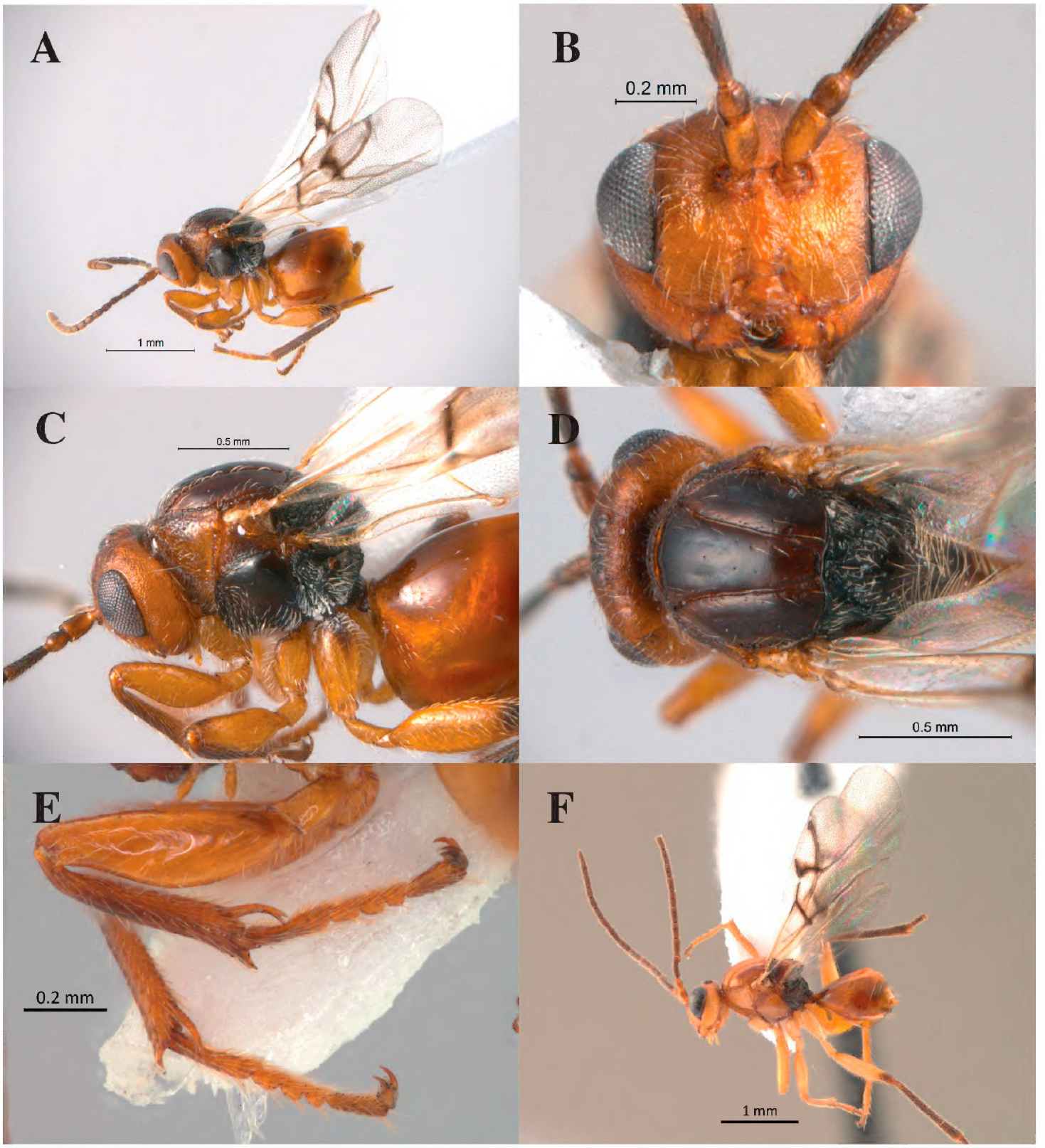

Figure 3. Specimens of the asexual generation of *B. mesoamericana*. Female (holotype): **A.** Habitus (lateral view); **B.** Head (frontal view); **C.** Mesosoma (lateral view); **D.** Mesosoma (dorsal view); **E.** Curved spine on the fore tibia. Paratype (male, sexual generation): **F.** Habitus (lateral view).

96°43'08.0"W; manual; 720 m; bosque de encino; Cyniver-47; A. Zaldívar, S. Fernández, S. Egan, R. Castañeda, J. Solis, cols • One female; Los Otates; sitio #1; 22/XI/2019; 19°30'48.2"N, 96°43'08.0"W; manual; 720 m; bosque de encino; Cyniver-48; A. Zaldívar, S. Fernández, S. Egan, R. Castañeda, J. Solis, cols • One female; Los Otates; sitio #1; 22/XI/2019; 19°30'48.2"N, 96°43'08.0"W; manual; 720 m; bosque de encino; Cyniver-49; A. Zaldívar, S. Fernández, S. Egan, R. Castañeda, J. Solis, cols • One female; Los Otates; sitio #1; 22/XI/2019; 19°30'48.2"N, 96°43'08.0"W; manual; 720 m; bosque de encino; Cyniver-50; A. Zaldívar, S. Fernández, S. Egan, R. Castañeda, J. Solis, cols.

Sexual generation • four females; same data as holotype; DNA voucher numbers CNIN5137, 38 [GenBank (COI) no. PZ044865; SRA (UCEs) accession nos SAMN55915580–81] • One female, five males; Veracruz, Municipio Actopan, Los Otates; 12/VI/2025; 19°30'36.7"N, 96°42'58.3"W; manual; 800 m; A. Zaldívar, J. Sols, E. Rangel, cols.; *Quercus oleoides*, agalla VIII, *Belonocnema* sexual de raíz tumorosa, aterciopelada, amarillento oscuro.

Diagnosis. *Belonocnema mesoamericana* sp. nov. is morphologically similar to its sister species, *B. kinseyi*, with both species having the spur on the anterior side of the fore tibia shorter than basitarsus and tibial spurs and the middle tibia without a spur in both generations (Fig. 3E). However, *B. mesoamericana* can be distinguished from *B. kinseyi* by its lateral propodeal carinae bent outwards (Fig. 4F) (straight in *B. kinseyi*), central propodeal area rugose (Fig. 4F) (punctate in *B. kinseyi*), and OOL 3.5× longer than LOL (Fig. 4E) (2.2× in *B. kinseyi*) in females of the sexual generation. The new species can also be distinguished from *B. kinseyi* by its lateral propodeal area rugose (alutaceous in *B. kinseyi*) and body color partially yellowish brown (Fig. 3A), with some specimens almost entirely dark brown (Fig. 1D) (always reddish brown in *B. kinseyi*) in most females of the asexual generation. Moreover, *B. mesoamericana* is the only species of the genus in which the rootlet galls formed by the sexual generation are velvety (Fig. 5C).

Description. Asexual female (Figs 1D, 3A–E): Body length 2.0–3.0 mm (N = 30). Yellowish to reddish brown, some specimens almost entirely dark brown; tip of mandibles, mesosoma (except pronotum), wing veins, anterior edge of meso, and metacoxae, distal edge of metafemur, fore, meso and meta tibia dark brown to black. Head finely coriaceous with sparse white setae; slightly rounded in dorsal view; 2.3× as broad as long in dorsal view; 1.4× as broad as long in frontal view; slightly broader than mesosoma. Gena finely coriaceous, not broadened behind eye in frontal view, equally broad as cross diameter of eye. Malar space finely coriaceous, without striae radiating from clypeus; eye 2.3× higher than length of malar space, malar sulcus present. Inner margins of eyes parallel. OOL 1.4× longer than POL; OOL 2.3× longer than LOL; ocelli ovate, all equal in size. Transfacial distance 1.7× longer than height of eye and 1.1× longer than height of lower face; diameter of antennal torulus 1.9× longer than distance between them, distance between torulus and eye margin 1.5× longer than diameter of torulus. Lower face finely coriaceous, with white setae, without striae radiating from clypeus, median area not elevated. Clypeus trapezoid, flat, broader than high, with deep anterior tentorial pits, distinct epistomal sulcus and clypeo-pleurostomal line. Frons finely coriaceous, sparsely pubescent; vertex, interocellar area, and occiput finely coriaceous. Postgena coriaceous, glabrous. Antenna 13 segmented, longer than head + mesosoma; F1 shorter than the length of scape + pedicel, 1.6× longer than F2. Mesosoma 1.2× longer than high in lateral view. Propleuron alutaceous, with short, sparse setae. Pronotum laterally striate finely rugose. Mesoscutum smooth, largely glabrous except for sparse setae on either side of notauli, alutaceous lateral to notaulus; longer than broad; notauli complete, deeply impressed for full length; median mesoscutal line absent; anterior parallel lines almost indistinct, parapsidal lines absent.

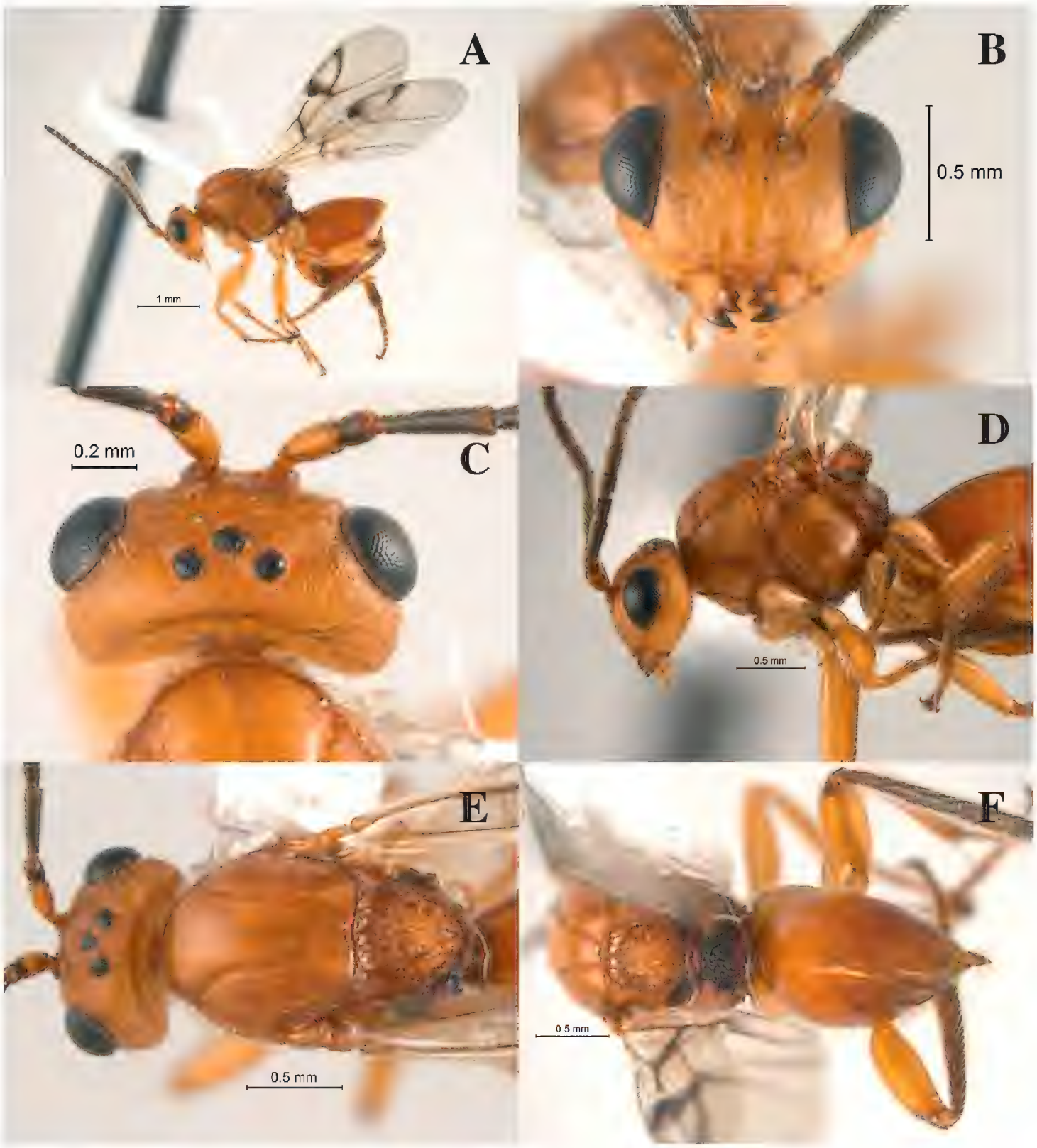

Figure 4. Specimens of the sexual generation of *B. mesoamericana*. Female (holotype): **A.** Habitus (lateral view); **B.** Head (frontal view); **C.** Head (dorsal view); **D.** Mesosoma (lateral view); **E.** Mesosoma (dorsal view); **F.** Mesosoma and metasoma (dorsal view).

Mesoscutellum as long as broad, slightly narrower posteriorly; distinctly shorter than mesoscutum, uniformly rugose, overhanging metanotum; scutellar foveae present, deep, smooth to slightly rugose, separated narrowly by carina. Mesopleural triangle with white sparse short setae, mesopleuron smooth, glabrous, with dense white setae along 2/3 of the way on the anterior and ventral margins. Lateral propodeal carinae distinct, bent outwards; central and lateral propodeal areas rugose, lateral propodeal area with dense white setae; nucha very short, coriaceous. Tibia setose on anterior edge; fore tibia prolonged on the anterior side into a curved spine, much shorter than tibial

Figure 5. Galls formed by *B. mesoamericana*. **A.** Galls induced by the asexual generation showing color variation from pale green to reddish. Galls induced by the sexual generation: **B.** After insect emergence; **C.** Previous to insect emergence, showing the velvety appearance.

spur and basitarsus; tarsal claws simple with a slight ridge but never a full tooth. Fore tibia with one and mid and hind tibia with two asymmetrical spurs. Fore wing hyaline, longer than body, margin with dense cilia; radial cell about 2× as long as wide; 2r infumated, Rs curved upwards and thickened at apex; radial cell open; areolet open and distinct; Rs + M reaching to M; cu-a absent; Cu1 broken. Metasoma as long as head + mesosoma, 1.2× longer than high in lateral view, smooth; second metasomal tergite setose laterally and medially; all subsequent tergites without setae, smooth, glossy; ventral spine of the hypopygium short, prominent part 1.7× as long as broad in ventral view, with white setae extending beyond the apex of spine.

Sexual female (Fig. 4A–F): Body length 3.4–4.0 mm (N = 6). Yellowish brown; pedicel, flagellomeres, tip of mandibles, central and sometimes lateral propodeal area, wing veins, hind tibia and tarsi dark brown. Head finely coriaceous with sparse white setae; slightly rounded in dorsal view; 2.2× as broad as long in dorsal view; 1.2× as broad as long in frontal view; slightly broader than mesosoma. Gena coriaceous, not broadened behind eye in dorsal view; 0.7 as broad as cross diameter of eye. Malar space coriaceous, without striae radiating from clypeus; eye 1.9× higher than length of malar space, malar sulcus present. Inner margins of eyes parallel. OOL 1.2× longer than POL; OOL 3.5× longer than LOL; ocelli ovate, all equal in size. Transfacial distance 1.5× longer than height of eye and 1.4× longer than height of lower face; diameter of antennal torulus about 2× longer than distance between them, distance between torulus and eye margin 1.2× longer than diameter of torulus. Lower face finely coriaceous-slightly rugulose, with white sparse setae, without striae radiating from clypeus, median area not elevated. Clypeus trapezoid, flat, broader than high, with deep anterior tentorial pits, distinct epistomal sulcus and clypeo-pleurostomal line. Frons finely coriaceous,

with sparse white setae; vertex, interocellar area and occiput finely coriaceous. Postgena coriaceous-finely rugose. Antenna 14 segmented, longer than head + mesosoma; F1 shorter than the length of scape + pedicel, 1.5× longer than F2. Mesosoma 1.3× longer than high in lateral view. Propleuron alutaceous, with sparse setae. Pronotum mostly smooth with fine striae. Mesoscutum mostly smooth, glabrous between notauli and sparse white setae along them, alutaceous lateral to notaulus; 1.1× longer than broad; notauli complete, deeply impressed for full length; median mesoscutal line almost indistinct, only weakly impressed in the posterior margin of mesoscutum; anterior parallel lines and parapsidal lines absent; mesoscutellum only slightly longer than broad, slightly narrower posteriorly; shorter than mesoscutum, uniformly rugose, overhanging metanotum; scutellar foveae deeply excavated, smooth to slightly rugose, fully delimited on all sides, separated narrowly by carina. Mesopleuron smooth, glabrous, with a few white setae along ventral and anterior margins; mesopleural triangle setose. Lateral propodeal carinae distinct, bent outwards, central propodeal area glabrous, rugose; lateral propodeal area alutaceous, with long white setae; nucha short, smooth. Tibiae setose, with long setae on anterior edge. Fore tibia with a single and middle and hind tibia with two spurs. Fore tibia prolonged on the anterior side into a curved spine, considerably shorter than tibial spur and basitarsus; spur of fore tibia one third the size of basitarsus. Middle tibia with a small, curved spine, shorter than tibial spurs. Tarsi setose. tarsal claws simple, with a slight basal ridge but never a full tooth. Fore wing hyaline, longer than body, margin with dense cilia; radial cell about 2.5× longer than wide; 2r infumated, Rs curved upwards and thickened at apex; radial cell open; areolet small and distinct; Rs + M reaching to M; cu-a absent; cu1 broken. Metasoma shorter than head + mesosoma, 1.2× longer than high in lateral view, smooth and glabrous; first and second metasomal tergites with sparse, short setae laterally; all subsequent tergites without setae, smooth, glossy. Ventral spine of the hypopygium short, prominent part 2.5× longer than broad in ventral view, with white setae extending beyond the apex of spine.

Male (Fig. 3F): Body length 2.6–3.0 mm (N = 5). Color slightly darker than sexual female; pedicel, lateral propodeal area and venter of mesopleuron dark brown to black. Sculptures like the sexual female. Antenna 15 segmented. F1 curved, excavated, and incised medially. Metasoma smaller than head + mesosoma.

Galls. Galls of the asexual generation are unilocular, pea-like, and occur on the ventral side of leaves (Fig. 5A). The number of galls varies from one to several, and they are irregularly arranged across the leaf. Gall color varies from pale green to reddish, becoming dark to greyish brown after insect emergence. Galls of the sexual generation, on the other hand, are globose, irregularly shaped, forming multilocular clusters on small rootlets. They have a velvety texture and are brown to light brown, turning paler and losing the velvety appearance after insect emergence (Fig. 5B, C). Rootlet galls formed by the sexual generation in the known species of *Belonocnema* can exhibit a lightly pubescent surface made of fine, short hairs in early growth that decreases as they mature. However, *B. mesoamericana* rootlet galls are densely pubescent throughout growth and maturation and the surface of the gall could be described as velvet-like.

A total of 332 and 22 specimens of *B. mesoamericana* were reared from galls formed by its asexual and sexual generations, respectively. Leaf galls of the asexual generation of *B. mesoamericana* were considerably abundant in the localities where they occurred. They were recorded from September to November, with most gallers and its associated hymenopterans emerging during late October to early November. Rootlet galls, on the other hand, appear to be scarcer and are considerably more difficult to find. We searched for this type of gall only in the locality of Los Otates during April in two consecutive years (2024–2025), finding them exclusively around a single tall tree near the soil surface. Further collections in other localities will allow to know the abundance of sexual galls in *B. mesoamericana*.

Plant host. The host plant of *B. mesoamericana*, *Q. oleoides*, is the most widespread oak species of the section Virentes, occurring from northern Mexico in Tamaulipas, to Costa Rica in Central America (Valencia 2004; Cavender-Bares et al. 2011, 2015). It has been reported in dry and tropical sub-deciduous forests at elevations that range from 150 to about 1000 m (Valencia, 2004).

Distribution. *Belonocnema mesoamericana* is currently only known from central Veracruz, Mexico. However, based on the geographic distribution of its oak host, its distribution is presumably broader.

Etymology. The name of the new species refers to the cultural-historical region situated in the Neotropics that extends from central Mexico to northwest Costa Rica, which mostly covers the geographic distribution of its host plant species, *Q. oleoides*.

Key to the asexual and sexual generations of *Belonocnema* species

The Zhang et al. (2021) key to the asexual and sexual generations of *Belonocnema* species is modified to include this new species:

1 Antennae with 14 (females) or 15 (males, F1 excavated) segments ..... **2 (sexual generation)**

– Antennae with 13 segments (Fig. 1A–D) ..... **5 (asexual generation)**

2 Spur on the anterior side of fore tibia longer than basitarsus and tibial spurs. Middle tibia with a smaller spur. Found on *Q. geminata* in Georgia and Florida ..... ***B. fossoria* Weld**

– Spur on the anterior side of fore tibia shorter than basitarsus, approximately the same length as tibial spurs. Middle tibia without a spur. Found on *Q. fusiformis*, *Q. virginiana*, or *Q. oleoides* **3**

3 Scutellar foveae shallow, weakly delimited posteriorly, separated broadly by a ridge (fig. 2F in Zhang et al. 2021). Rs vein thickened and infumated. Found east of Gautier, Mississippi, associated predominantly with *Q. virginiana* and rarely with *Q. geminata* ..... ***B. treatae* Mayr**

– Scutellar foveae deeply excavated, delimited on all sides, separated narrowly by a carina (Fig. 4E). Rs vein thickened but not infumated. Found west of Gautier, Mississippi **4**

4 Lateral propodeal carinae straight; central propodeal area punctate; OOL about 2.2× LOL. Found west of Gautier, Mississippi (30.3858°N, 88.6117°W). Rootlet galls smooth, not velvety. Associated with *Q. fusiformis* and *Q. virginiana*..... ***B. kinseyi* Weld**

– Lateral propodeal carinae bent outwards; central propodeal area rugose; OOL about 3.5× LOL. Rootlet galls velvety (Fig. 5C). Associated with *Q. oleoides* ....***B. mesoamericana* Zhang, Zaldívar-Riverón & Fernández-Flores, sp. nov.**

5 Spur on the anterior side of fore tibia longer than basitarsus and tibial spurs (fig. 3A in Zhang et al. 2021). Middle tibia with a smaller spur. Fore wing small and curved (Fig. 1A), areolet absent. Found on *Q. geminata* in Georgia and Florida ..... ***B. fossoria***

– Spur on the anterior side of fore tibia shorter than basitarsus, approximately the same length as tibial spurs (Fig. 3E). Middle tibia without a spur. Fore wing large and not curved (Figs 1B–D, 3A), areolet present (could be small and indistinct). Found on *Q. fusiformis*, *Q. virginiana*, or *Q. oleoides*..... **6**

6 Yellowish brown in color (Fig. 1C), areolet in fore wing large and distinct. Found east of Gautier, Mississippi, associated predominantly on *Q. virginiana* and rarely with *Q. geminata*..... ***B. treatae***

– Reddish brown to partially yellowish brown or dark brown in color (Figs 1B, D, 3A), areolet in fore wing small and indistinct..... 7

7 Body reddish brown (Fig. 1B); lateral propodeal area alutaceous. Found west of Gautier, Mississippi, associated with *Q. fusiformis* and *Q. virginiana*..... ..... ***B. kinseyi***

– Body partially yellowish brown to dark brown (Figs 1D, 3A); lateral propodeal area rugose. Associated with *Q. oleoides*..... ***B. mesoamericana* sp. nov.**

Associated insect community

A list of the hymenopteran morphospecies reared from galls of the asexual and sexual generations of *B. mesoamericana*, including their respective families and genera, is provided in Table 2. Digital photographs of representative hymenopteran taxa reared from galls of *B. mesoamericana* are provided in Fig. 6A–H.

Overall, a considerably higher diversity and abundance of hymenopteran taxa were reared from the galls induced by the asexual generation of *B. mesoamericana* compared with those reared from its sexual generation, which is similar to patterns observed in other *Belonocnema* species in the U.S. (Forbes et al. 2016). A total of 43 hymenopteran morphospecies were obtained from the two types of *B. mesoamericana* galls, with 41 reared from galls formed by the sexual generation and three from those of the asexual generation, with only one species (*Ormyrus* sp. 1) being found in galls of both generations (Table 2). These morphospecies belong to 13 families and 15 identified genera. The genera with the highest numbers of reared morphospecies were *Ormyrus* Westwood (Ormyridae) with six, *Synergus* Hartig (Cynipidae) and *Sycophila* Walker (Eurytomidae) with four, and *Eprhopalotus* Girault (Eulophidae), *Eurytoma* Illiger (Eurytomidae), and *Torymus* Dalman (Torymidae)

Table 2. List of hymenopteran families, genera, morphospecies and number of specimens reared from galls formed by the asexual and sexual generations of *B. mesoamericana*, including their presumable biology. P= parasitoid; I= inquiline. Without asterisk= reared from galls formed by asexual generation; *= reared from galls formed by sexual generation.

Family	Genus	Morphospecies	Probable biology	No. specimens
Bethylidae	Undetermined	sp.	P	1
Braconidae	*Bracon*	sp. 1	P	1
Braconidae	*Bracon*	sp. 2	P	2
Braconidae	*Allorhogas*	sp.	P/I?	3
Cynipidae	*Synergus*	sp. 1	I	231
Cynipidae	*Synergus*	sp. 2	I	53
Cynipidae	*Synergus*	sp. 3*	I	3
Encyrtidae	Undetermined	sp.	P	1
Trichogrammatidae	*Trichogramma*	sp.	P	1
Eulophidae	*Eprhopalotus*	sp. 1	P	7
Eulophidae	*Eprhopalotus*	sp. 2	P	6
Eulophidae	*Eprhopalotus*	sp. 3	P	250
Eulophidae	*Galeopsomyia*	sp.	P	18
Eulophidae	*Paracrias*	sp.	P	4
Eupelmidae	*Brasema*	sp. 1	P	12
Eupelmidae	*Brasema*	sp. 2	P	2
Eupelmidae	*Brasema*	sp. 3	P	10
Eupelmidae	*Brasema*	sp. 4	P	2
Eurytomidae	*Sycophila*	sp. 1	P	40
Eurytomidae	*Sycophila*	sp. 4	P	39
Eurytomidae	*Sycophila*	sp. 2	P	6
Eurytomidae	*Sycophila*	sp. 3	P	10
Eurytomidae	*Phylloxeroxenus*	sp.	P	13
Eurytomidae	*Tenuipetiolus*	sp.	I/P?	118
Eurytomidae	*Eurytoma*	sp. 1	I/P?	30
Eurytomidae	*Eurytoma*	sp. 2	I/P?	40
Ormyridae	*Ormyrus*	sp. 1	P	29
Ormyridae	*Ormyrus*	sp. 1*	P	5
Ormyridae	*Ormyrus*	sp. 2	P	1
Ormyridae	*Ormyrus*	sp. 3	P	2
Ormyridae	*Ormyrus*	sp. 4	P	1
Ormyridae	*Ormyrus*	sp. 5	P	1
Ormyridae	*Ormyrus*	sp. 6	P	1
Pteromalidae	Pteromalini	–	P	2
Pteromalidae	Pteromalini	–	P	1
Pteromalidae	Pteromalini	–	P	2
Pteromalidae	Pteromalini	–	P	2
Scelionidae	Undetermined	–	P	2
Signiphoridae	*Signiphora*	sp.	P	1
Torymidae	*Torymus*	sp. 1	P	12
Torymidae	*Torymus*	sp. 2	P	2
Torymidae	*Torymus*	sp. 3	P	3
Torymidae	*Torymus*	sp. 4*	P	124

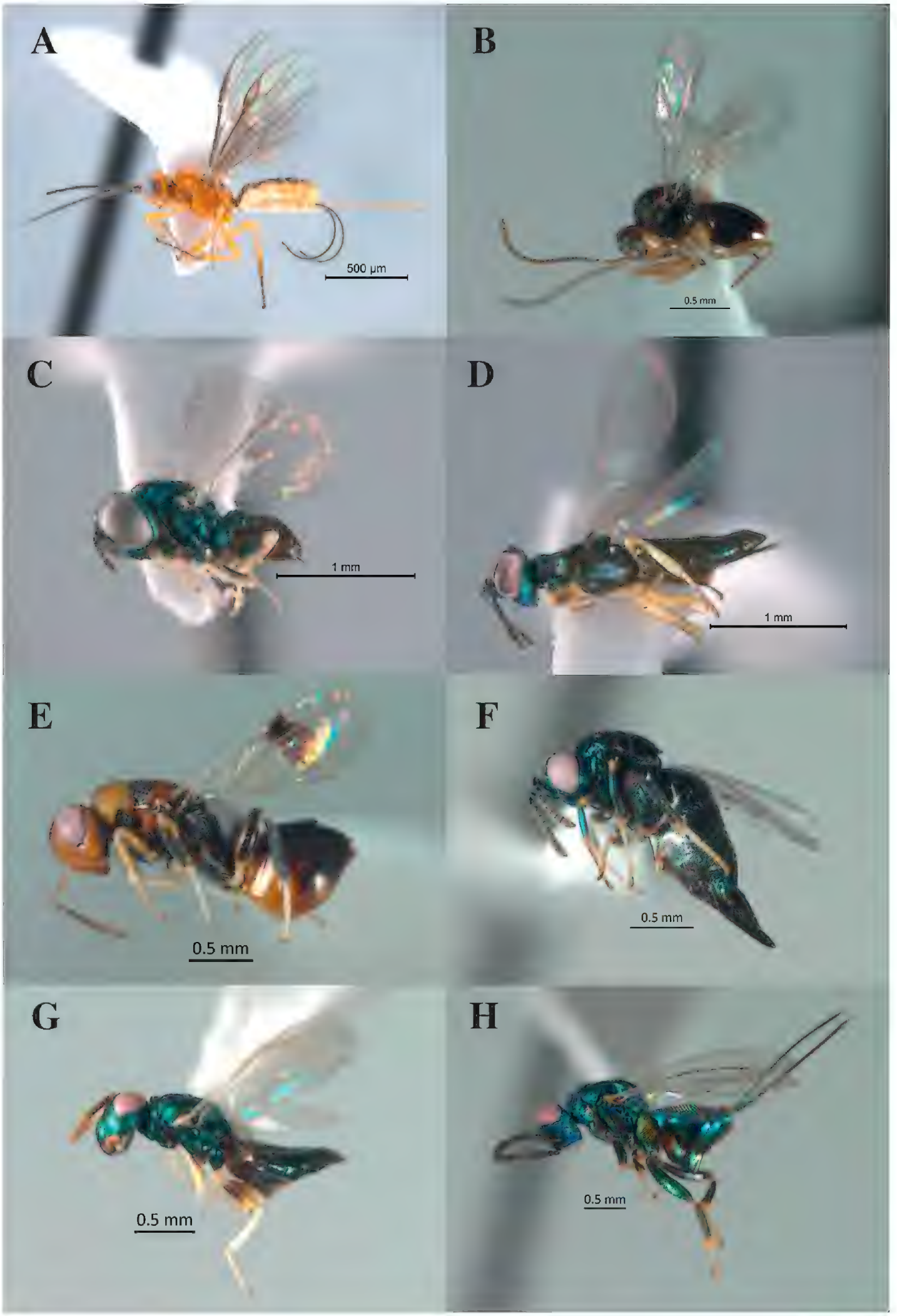

Figure 6. Representative hymenopteran taxa associated with galls of *B. mesoamericana*: **A.** *Bracon* sp. 2 (Braconidae); **B.** *Synergus* sp. 2 (Cynipidae); **C.** *Eprhopalotus* sp. 3 (Eulophidae); **D.** *Brasema* sp. 3 (Eupelmidae); **E.** *Sycophila* sp. 3 (Eurytomidae); **F.** *Ormyrus* sp. 1 (Ormyridae); **G.** Pteromalini sp. 3 (Pteromalidae); **H.** *Torymus* sp. 1 (Torymidae).

with three morphospecies each. *Synergus*, *Eprhopalotus* and *Eurytoma* each included morphospecies reared from galls of the asexual generation that yielded the highest numbers of specimens, with 231, 250, and 118 individuals, respectively.

Discussion

Here we have conducted an integrative systematic study of a new species of *Belonocnema* that induces galls on *Q. oleoides* in the state of Veracruz, Mexico. This live oak is widely distributed across the Mesoamerican region, from northern Mexico to Costa Rica in Central America (Cavender-Bares et al. 2011, 2015), and we therefore expect that the new species has a broader geographic distribution in additional northern and southern regions where its host plant occurs. In Mexico, *Q. oleoides* is mainly distributed across the Sierra Madre Oriental, mainly in its eastern slopes, in the states Hidalgo, Puebla, San Luis Potosí, Tamaulipas and Veracruz, but also occurs in the Sierra Madre del sur in the states of Chiapas and Oaxaca, as well as in Campeche, Tabasco and Yucatan (Valencia 2004).

Integrative taxonomy

We examined complementary lines of evidence for the recognition of the new species, including external morphological and gall features, as well as mitochondrial DNA barcoding and nuclear genome-scale (UCE) data. The gathered morphological and molecular information helped to delimit *B. mesoamericana*, despite it being morphologically and genetically similar to its sister species, *B. kinseyi*. This emphasizes the importance of conducting integrative taxonomic studies (*sensu* Padial et al. 2010), particularly in morphologically conserved, highly speciose taxa such as members of the family Cynipidae.

Belonocnema mesoamericana shares various external morphological features with *B. kinseyi*, which has a contiguous distribution in northern Mexico, as it induces galls on *Q. fusiformis*, whose geographic distribution extends southwards to Tamaulipas in northern Mexico (Zhang et al. 2021). Among the potential morphological synapomorphies shared between the latter two species are a spur on the anterior side of the fore tibia shorter than basitarsus and approximately the same length as tibial spurs, and the middle tibia without a spur. Future collections in northern Mexico will reveal whether these two species overlap in their geographic distributions and may have experienced introgression.

Generation of DNA barcoding data in some cases resulted in genetic distances marginally lower than 2% between examined specimens of *B. mesoamericana* and *B. kinseyi*, which is the minimum distance commonly observed among species of various animal taxa (Hebert et al. 2003). Barcoding distances lower than the 2% threshold, however, are frequent in recently diverged species (van Velzen et al. 2012). The nuclear UCE marker, on the other hand, recovered the members of the two species

as reciprocally monophyletic with strong support (Fig. 2). This might be the result of mitonuclear discordance, including the closely related *B. fossoria* and *B. treatae* also cannot be accurately separated using COI despite clear nuclear and morphological differences (Zhang et al. 2021).

Associated hymenopteran community

The number of hymenopteran species and the phylogenetic diversity associated with the galls of both generations of *B. mesoamericana* (43 morphospecies belonging to 13 families) was higher than that reported in a previous study of *B. treatae* in the U.S. (Forbes et al. 2016), which identified 21 species from 10 families. Galls of the asexual generation of *B. mesoamericana* also had a higher hymenopteran species richness than those of the asexual generation of two other gall forming cynipid species of *Quercus* species of the section Virentes, *Bassettia pallida* Ashmead and *Disholcaspis quercusvirens* Ashmead, with 21 and nine species, respectively (Bird et al. 2013; Weinersmith et al. 2020).

The considerably higher species richness observed in the galls of the new species described here is remarkable, considering that most of our sampling was restricted to a single known host oak species and to only one region in the state of Veracruz, Mexico, compared with the broader sampling carried out for *B. treatae* on its two host oak species, *Q. fusiformis* and *Q. virginiana*. Further barcoding studies including sequencing specimens of all identified morphospecies will delimit with more confidence the number of hymenopteran species associated with the galls of *B. mesoamericana*. Similarly to *B. treatae*, the galls of the asexual generation of *B. mesoamericana* harbored most of the identified hymenopteran species richness, with 41 species, compared with only three species found in its sexual generation. This could be due to different factors such as the timing of oviposition, onset of gall growth, development period, accessibility of the natural enemies, and gall structure (Forbes et al. 2016; Ward et al. 2022b).

Based on the recorded biology of the identified hymenopteran genera, the species diversity found in the galls of *B. mesoamericana* is mainly represented by parasitoids of this gall former, but also includes hyperparasitoids, parasitoids of associated species from other insect orders, as well as inquilines that feed on gall tissue (e.g. *Synergus*, probably *Allorhogas* Gahan) (Ward et al. 2022b). Interestingly, among all these reared species, only one presumable parasitoid (*Ormyrus* sp. 1) was found in both generations. This reflects the complex biologies of the members of this community and suggests that several of them could be generalist parasitoids forming part of the community associated with some of the remaining nine types of galls induced by Cynipini species that we found on *Q. oleoides* during our field trips carried out in the state of Veracruz, Mexico, or that they may even be part of other insect systems. Regarding the species that are not known to be directly associated with the gall forming cynipids or their parasitoid or inquilines, this could be the case for some taxa, such as *Bracon* Fabricius (Braconidae), *Signiphora* Ashmead (Signiphoridae), *Trichogramma* Westwood (Trichogrammatidae), and the unidentified species of Encyrtidae, Bethylidae and Scelionidae. In these cases, they might be attacking secondary occupants or inquilines of other

insect orders, such as lepidopteran and coleopteran species (Ward et al. 2022b). The use of novel molecular techniques for confirming insect associations (Muñoz-Sánchez et al. manuscript in review) will reveal the degree of host specialization of the recorded natural enemies of *B. mesoamericana*, as well as the level of overlap between insect communities associated with the Cynipini species present in *Q. oleoides*.

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